CLAIMS

What is claimed is:

1. A promoter insertion construct for identifying a gene whose product modulates a specific control phenotype, said promoter insertion construct comprising:

a promoter element, a downstream recognition site for a disrupting agent having a site specific recombinase activity, and an upstream recognition site for the disrupting agent;

wherein the recognition sites are configured such that treatment of a DNA molecule comprising said promoter insertion construct and flanking downstream and upstream DNA sequences results in removal of the promoter from the DNA molecule; and

wherein said promoter insertion construct lacks a downstream termination sequence, a splice donor sequence, and a splice acceptor sequence.

2. The promoter insertion construct of claim 1, wherein the promoter insertion construct comprises

a) a promoterless marker gene that is downstream of and operably linked with the promoter element and an internal ribosome entry site downstream of the promoterless marker gene, or

b) a marker gene element that is upstream of the promoter element, said marker gene element comprising a marker gene, and a second promoter element for promoting expression of the marker gene product, or

c) both a and b.

3. The promoter insertion construct of claim 1, wherein the disrupting agent is a recombinase.

4. The promoter insertion construct of claim 1, wherein the disrupting agent is a transposase.

5. A method of identifying a gene whose product modulates a control phenotype of interest, comprising:

introducing the promoter insertion construct of claim 1 into the genomes of a collection of host cells having the control phenotype of interest to provide a population of mutagenized cells;

selecting mutagenized cells exhibiting a mutant phenotype of interest to provide a pool of mutant cells;

treating the mutant cells with a disrupting agent having recombinase activity;

detecting changes or the lack thereof in the linkage between the promoter element and downstream host genomic DNA sequences in treated, mutant cells, or in treated mutant cells and untreated, mutant cells;

correlating changes or lack thereof in the linkage between the promoter element and the downstream host genomic DNA sequences in treated mutant cells, or in both untreated and treated mutant cells, with the phenotypes of said cells;

wherein a host genomic DNA fragment that

a) is operably linked with the promoter element in untreated, mutant cells that display the mutant phenotype, but is not operably linked with the promoter element in treated cells that display the control phenotype, or

b) is operably linked with the promoter insertion construct in treated cells that maintain the mutant phenotype, but is not operably linked with the promoter insertion construct in treated cells that display the control phenotype, or

c) both a and b.

encodes a product that modulates the control phenotype of interest.

6. The method of claim 5, wherein the changes or lack thereof in the linkage between the promoter element and the downstream host genomic DNA sequences is detected by sequencing.

7. The method of claim 5, wherein the percentage of mutant cells that spontaneously revert to the control phenotype without treatment with the disrupting agent is compared to the percentage of mutant cells that revert to the control phenotype after treatment with the disrupting agent.

8. The method of claim 5, wherein the promoter insertion construct further comprises: one or more marker genes, and

wherein the method further comprises:

i) identifying mutagenized cells by monitoring expression of the marker gene;

ii) identifying treated, mutant cells in which the linkage between the promoter element and the downstream host genomic sequence has been disrupted by monitoring expression of the marker gene; and

iii) identifying treated, mutant cells in which the linkage between the promoter element and the downstream host genomic sequence has been maintained by monitoring expression of the marker gene.

iv) or any combination of steps i, ii, and iii.

9. The method of claim 5, wherein said host cells comprises a selection system comprising:

one or more constructs comprising a second promoter element and at least one marker gene that is operably linked with the second promoter element, wherein said second promoter element promotes expression of an RNA or protein that is associated with the control phenotype.

10. A promoter insertion construct for identifying a gene whose product modulates a control phenotype of interest, comprising:

a promoter element and a downstream recognition site for a disrupting agent having recombinase activity, wherein said construct lacks a termination sequence, a splice donor sequence, and a splice acceptor sequence, and

wherein the disrupting agent is a recombinase, and a circular double-stranded DNA molecule comprising

a recognition site for the recombinase, wherein said circular double-stranded DNA molecule lacks a promoter for promoting expression of the marker gene, a splice donor site, a splice acceptor site and a termination sequence.

11. The promoter insertion construct of claim 10, wherein said circular double-stranded DNA molecule further comprises a promoterless marker gene.

12. A method of identifying a gene whose product modulates a control phenotype of interest, comprising:

introducing the promoter insertion construct of claim 10 into the genomes of a collection of host cells having the control phenotype of interest to provide a population of mutagenized cells;

selecting mutagenized cells exhibiting a mutant phenotype to provide a pool of mutant cells;

treating the mutant cells with the disrupting agent;

detecting changes or the lack thereof in the linkage between the promoter element and downstream host genomic DNA sequences in treated mutant cells, or in both treated and untreated mutant cells;

correlating the changes or lack thereof in the linkage between the promoter element and the downstream host genomic DNA sequences in treated mutant cells, or in both untreated and treated mutant cells, with the phenotypes of said cells;

wherein a host genomic DNA fragment that

a) is operably linked with the promoter element in untreated, mutant cells that display the mutant phenotype, but is not operably linked with the promoter element in treated cells that display the control phenotype, or

b) is operably linked with the promoter insertion construct in treated cells that maintain the mutant phenotype, but is not operably linked with the promoter insertion construct in treated cells that display the control phenotype, or

c) both a and b.

encodes a product that modulates the control phenotype of interest.

13. The method of claim 12, wherein the percentage of mutant cells that spontaneously revert to the control phenotype without treatment with the disrupting agent is compared to the percentage of mutant cells that revert to the control phenotype after treatment with the disrupting agent.

14. The method of claim 12, wherein said host cells comprise a selection system comprising:

one or more constructs comprising a second promoter element and at least one marker gene that is operably linked with the second promoter element, wherein said second promoter element promotes expression of an RNA or protein that is associated with the control phenotype.

15. A promoter insertion construct for identifying a gene whose product modulates a specific control phenotype, said promoter insertion construct comprising:

a promoter element, a downstream recognition site for a disrupting agent having site specific recombinase activity, an upstream recognition site for the disrupting agent; and an RNA polyadenylation sequence or a transcription terminator sequence, or both upstream of the upstream recognition site;

wherein the recognition sites are configured such that treatment of a DNA molecule comprising said promoter insertion construct and flanking downstream and upstream DNA sequences results in inversion of the promoter within the DNA molecule; and

wherein said promoter insertion construct lacks a downstream termination sequence, a splice donor sequence, and a splice acceptor sequence.

16. The promoter insertion construct of claim 15, wherein the promoter insertion construct comprises

a) a promoterless marker gene that is downstream of and operably linked with the promoter element and an internal ribosome entry site downstream of the promoterless marker gene,

b) a promoterless marker gene that is upstream of the upstream recognition site and, optionally, an internal ribosome entry site upstream of the promoterless marker gene;

c) a marker gene element that is upstream of the promoter and downstream of the upstream recognition site, or

d) any combination of a, b, and c.

17. A method of identifying a gene whose product modulates a control phenotype of interest, comprising:

introducing the promoter insertion construct of claim 14 into the genomes of a collection of host cells having the control phenotype of interest to provide a population of mutagenized cells;

selecting mutagenized cells exhibiting a mutant phenotype to provide a pool of mutant cells;

treating the mutant cells with a disrupting agent having recombinase activity;

detecting changes or the lack thereof in the linkage between the promoter element and downstream host genomic DNA sequences in treated mutant cells, or in both treated and untreated mutant cells;

correlating the changes or lack thereof in the linkage between the promoter element and the downstream host genomic DNA sequences treated mutant cells, or in both untreated and treated mutant cells, with the phenotypes of said cells;

wherein a host genomic DNA fragment that

a) is operably linked with the promoter element in untreated, mutant cells that display the mutant phenotype, but is not operably linked with the promoter element in treated cells that display the control phenotype, or

b) is operably linked with the promoter insertion construct in treated cells that maintain the mutant phenotype, but is not operably linked with the promoter insertion construct in treated cells that display the control phenotype, or

c) both a and b.

encodes a product that modulates the control phenotype of interest.

18. The method of claim 17, wherein the promoter insertion construct further comprises one or more marker genes or promoterless marker genes, and

wherein the method further comprises:

i) identifying mutagenized cells by monitoring expression of the marker gene;

ii) identifying treated, mutant cells in which the linkage between the promoter element and the downstream host genomic sequence has been disrupted by monitoring expression of the marker gene; and

iii) identifying treated, mutant cells in which the linkage between the promoter element and the downstream host genomic sequence has been maintained by monitoring expression of the marker gene, or

iv) any combination of i, ii, and iii.

19. A promoter insertion construct for identifying a gene whose product modulates a specific control phenotype, said promoter expression construct comprising:

a promoter element, a downstream recognition site for a disrupting agent having recombinase activity, an upstream recognition site for the disrupting agent, a promoterless marker gene that is downstream of and operably linked to the promoter element; and an internal ribosome entry site that is downstream of the promoterless marker gene;

wherein said promoter insertion construct lacks a downstream termination site.

20. The promoter insertion construct of claim 19, wherein said construct comprises:

a) a translation start site, and

b) an uncoupled splice donor site.

21. The promoter insertion construct of claim 19, wherein the recognition sites are configured such that treatment of a DNA molecule comprising said promoter insertion construct and flanking downstream and upstream DNA sequences results in removal of the promoter from the DNA molecule

22. The promoter insertion construct of claim 18, wherein the construct comprises an RNA polyadenylation sequence, or a transcription terminator sequence, or both, upstream of the upstream recognition site; and

wherein the recognition sites are configured such that treatment of a DNA molecule comprising said promoter insertion construct and flanking downstream and upstream DNA sequences results in inversion of the promoter within the DNA molecule

23. A method of identifying a gene whose product modulates a control phenotype of interest, comprising:

introducing the promoter insertion construct of claim 18 into the genomes of a collection of host cells having the control phenotype of interest to provide a population of mutagenized cells;

selecting mutagenized cells exhibiting a mutant phenotype to provide a pool of mutant cells;

treating the mutant cells with a disrupting agent having recombinase activity;

detecting changes or the lack thereof in the linkage between the promoter element and downstream host genomic DNA sequences in treated mutant cells, or in both treated and untreated mutant cells;

correlating the changes or lack thereof in the linkage between the promoter element and the downstream host genomic DNA sequences in treated mutant cells, or in both untreated and treated mutant cells, with the phenotypes of said cells;

wherein a host genomic DNA fragment that

a) is operably linked with the promoter element in untreated, mutant cells that display the mutant phenotype, but is not operably linked with the promoter element in treated cells that display the control phenotype, or

b) is operably linked with the promoter insertion construct in treated cells that maintain the mutant phenotype, but is not operably linked with the promoter insertion construct in treated cells that display the control phenotype, or

c) both a and b.

encodes a product that modulates the control phenotype of interest.

24. The method of claim 23, wherein the disrupting agent is a recombinase.

25. The method of claim 23, wherein the disrupting agent is a transposase.

26. A promoter insertion construct set for identifying a gene whose product modulates a specific control phenotype, said promoter insertion construct set comprising three different promoter insertions constructs, wherein each of said three different promoter insertion constructs comprise:

a promoter element;

a first recognition site for a disrupting agent having recombinase activity, said first recognition site being upstream of the promoter element;

a second recognition site for the disrupting agent, said second recognition site being downstream of the promoter element;

an internal ribosome entry site downstream of the promoter element;

a translation start site downstream of the internal ribosome entry site;

an open reading frame sequence downstream of the translation start site and operably linked with the promoter element; and

an uncoupled splice donor site downstream of the of the open reading frame sequence;

wherein said promoter insertion construct lacks a downstream termination sequence and a splice acceptor sequence; and

wherein the uncoupled splice donor sites in the three different promoter insertion constructs are positioned in a different reading frame with respect to the translation start site.

27. The promoter insertion construct set of claim 26, wherein each of said constructs comprises a promoterless marker gene downstream and operably linked to the promoter element and an internal ribosome entry site downstream of the promoterless marker gene.

28. The promoter insertion construct set of claim 26, wherein each of said constructs is in a retrovirus-based vector or a transposon-based vector.

29. A method of identifying a gene whose product modulates a specific control phenotype, comprising:

introducing the promoter insertion construct set of claim 26 into the genomes of a collection of host cells having the control phenotype of interest to provide a population of mutagenized cells;

selecting mutagenized cells exhibiting a mutant phenotype to provide a pool of mutant cells;

treating the mutant cells with a disrupting agent having recombinase activity;

detecting changes or the lack thereof in the linkage between the promoter element and downstream host genomic DNA sequences in treated mutant cells, or in both treated and untreated mutant cells;

correlating changes or lack thereof in the linkage between the promoter element and the downstream host genomic DNA sequences in treated mutant cells, or in both untreated and treated mutant cells, with the phenotypes of said cells;

wherein a host genomic DNA fragment that

a) is operably linked with the promoter element in untreated, mutant cells that display the mutant phenotype, but is not operably linked with the promoter element in treated cells that display the control phenotype, or

b) is operably linked with the promoter insertion construct in treated cells that maintain the mutant phenotype, but is not operably linked with the promoter insertion construct in treated cells that display the control phenotype, or

c) both a and b.

encodes a product that modulates the control phenotype of interest.

30. The method of claim 29, wherein the promoter insertion construct further comprises one or more marker genes or promoterless marker genes, and

wherein the method further comprises:

i) identifying mutagenized cells by monitoring expression of the marker gene;

ii) identifying treated, mutant cells in which the linkage between the promoter element and the downstream host genomic sequence has been disrupted by monitoring expression of the marker gene;

iii) identifying treated, mutant cells in which the linkage between the promoter element and the downstream host genomic sequence has been maintained by monitoring expression of the marker gene; or

iv) any combination of a, b, and c.

31. A promoter insertion construct set for identifying a gene whose product modulates a control phenotype of interest, said set comprising three different promoter insertion constructs, each of said promoter insertion constructs, comprising:

a promoter element;

a downstream recognition site for a disrupting agent having recombinase activity;

an internal ribosome entry site downstream of the promoter element;

a translation start site downstream of the internal ribosome entry site;

an open reading frame sequence downstream of the translation start site and operably linked with the promoter element; and

an uncoupled splice donor site downstream of the of the open reading frame sequence;

wherein the uncoupled splice donor sites in the three different promoter insertion constructs are positioned in a different reading frame with respect to the translation start site;

wherein said construct lacks a termination sequence; and

wherein the disrupting agent is a recombinase, and a circular double-stranded DNA molecule comprising:

a recognition site for the recombinase.

32. The promoter insertion construct set of claim 31, wherein the plasmid comprises a promterless marker gene.

33. A method of identifying a gene whose product modulates a specific control phenotype, comprising:

introducing the promoter insertion construct set of claim 31 into the genomes of a collection of host cells having the control phenotype of interest to provide a population of mutagenized cells;

selecting mutagenized cells exhibiting a mutant phenotype to provide a pool of mutant cells;

treating the mutant cells with a disrupting agent having recombinase activity;

detecting changes or the lack thereof in the linkage between the promoter element and downstream host genomic DNA sequences in treated mutant cells, or in both untreated and treated mutant cells;

correlating the changes or lack thereof in the linkage between the promoter element and the downstream host genomic DNA sequences in treated mutant cells, or in both the untreated and treated mutant cells, with the phenotypes of said cells;

wherein a host genomic DNA fragment that

a) is operably linked with the promoter element in untreated, mutant cells that display the mutant phenotype, but is not operably linked with the promoter element in treated cells that display the control phenotype, or

b) is operably linked with the promoter insertion construct in treated cells that maintain the mutant phenotype, but is not operably linked with the promoter insertion construct in treated cells that display the control phenotype, or

c) both a and b.

encodes a product that modulates the control phenotype of interest.

34. The methods of claims 5, 12, 17, 23, 29, and 33, wherein multiple copies of the promoter insertion construct are inserted into the genome of each of the host cells.

35. A recombinant cell for identifying genes whose products modulate a select biological process, comprising:

the promoter insertion construct of claims 1, 10, 15, 19, or 26 wherein said promoter insertion construct is integrated into the cell's genome.

36. The recombinant cell of claim 35, wherein multiple copies of the promoter insertion construct are integrated into the cell's genome.

37. The recombinant cell of claim 35, wherein the cell comprises a selection system comprising:

one or more constructs comprising a second promoter element and at least one marker gene that is operably linked with the second promoter element, wherein said second promoter element promotes expression of an RNA or protein that is associated with the control phenotype.

38. The recombinant cell of claim 35 wherein the marker gene is a selectable marker gene.

39. The recombinant cell of claim 35, wherein the second promoter element is operably linked with a positive selectable marker gene, or a negative selectable marker gene, or both.